function in executing fast escape, however, the Mauthner system has been evolutionarily malleable, having been incorporated into a range of modified C-start behaviours. For example, goldfish use C-starts during prey capture as well as predator avoidance. Similarly, archer fish have evolved a dramatic prey capture mechanism, whereby the retrieval of insects dislodged from vegetation by a spit of water involves a C-start with the hallmarks of Mauthner cell involvement. Recent evidence suggests that flying fish become airborne using an adapted system in which Mauthner cells connect to fin adductor motorneurons. In contrast to C-starts, however, left and right fin motorneurons are activated simultaneously, producing a sufficiently powerful bilateral fin adduction for an aerial escape. A similar adaptation occurs in the Mauthner cell system of anuran amphibians. Whilst in larval stages the Mauthner cells mediate classical C-starts, the cells atrophy as the tail regresses during metamorphosis but are retained in limbed juveniles to mediate a powerful, synchronous contraction of the two hind legs in a diving startle response which propels them away from danger.

In conclusion, Mauthner cells have evolved to maximize the speed of escape and hence optimize survival. During evolution, the Mauthner system has become incorporated into modified escape and predatory behaviours suiting the morphological, behavioural and ecological constraints of the host organism.

Where can I find out more?

Cachope, R., Mackie, K., Triller, A., O'Brien, J., and Pereda, A. (2007). Potentiation of electrical and chemical synaptic transmission mediated by endocannabinoids. Neuron *56*, 1034–1037.

Eaton, R.C., Lee, R.K.K., and Foreman, M.B. (2001). The Mauthner cell and other identified neurons of the brainstem escape network of fish. Prog. Neurobiol. *63*, 467–485.

Faber, D.S., and Korn, H. (1978). Neurobiology of the Mauthner Neuron. Raven Press.

Fetcho, J. (1991). The spinal network of the Mauthner cell. Brain Behav. Evol. *37*, 298–316.

Korn, H., and Faber, D.S. (2005). The Mauthner cell half a century later: a neurobiological model for decision making. Neuron *47*, 13–28.

Liu, K.S., and Fetcho, J.R. (1999). Laser ablations reveal functional relationships of segmental hindbrain neurons in zebrafish. Neuron *23*, 325–335.

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Primer

Orchestration of the immune response by dendritic cells

Matthew R. Buckwalter and Matthew L. Albert*

The immune system is arguably one of the most complex cellular organizations that exists in the body. This system is composed of multiple cell types that are arranged in distinct organs or circulate through the blood and peripheral tissues. The complexity of the immune system is not superfluous, but rather it is required to fulfill the multifaceted purpose of the immune system, namely: the recognition of the diverse repertoire of micro-organisms; the detection of neoplastic legions originating from a range of tissues; and, while executing these tasks, the maintenance of peripheral tolerance by suppressing detrimental responses against healthy tissues. Dendritic cells are critical players in conducting the immune response to fulfill these roles. Here we provide an overview of how dendritic cells monitor their surrounding environment and coordinate an appropriate response during both steady-state and inflammatory conditions. We also highlight some of the current approaches aimed to harness the unique properties of these cells for use as therapeutic agents against cancer and infectious disease.

Discovery

The term 'dendritic' was first used by Ralph Steinman and Zanvil Cohn in 1973 to describe a novel cell type identified in the secondary lymphoid organs of mice. Using microscopy techniques, they characterised this relatively rare population (~1%) on the basis of its adherence properties and morphology, with the most striking feature being its long cytoplasmic processes, which extend and retract from the cell body. A physiological role for this newly discovered cell type was not immediately appreciated. It was several years before dendritic cells were identified as 'accessory cells', which demonstrated a capacity, greater than that of macrophages, to stimulate allogeneic lymphocytes. Another important milestone in the understanding of dendritic cell biology was the discovery that these cells have the ability to present antigen on major histocompatibility complex (MHC) class I and II molecules. Upon migration and maturation, dendritic cells become capable of engaging lymphocytes and initiating immune programs. These observations led to dendritic cells being regarded as the 'sentinels' of the immune system and also as 'professional' antigen-presenting cells, for their multiple roles in orchestrating immunity.

Dendritic cell subsets

Dendritic cells have been categorized into multiple subsets with the two broadest categories being conventional dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs). cDCs can be further subdivided into distinct populations on the basis of their origin, location and differential expression of surface markers. For example, multiple subtypes of cDC exist in the skin: the cDCs in the epidermis are termed Langerhans cells and possess unique structures called Birbeck granules; both Langerhans cells and dermal dendritic cells (also called interstitial dendritic cells) are present in the dermal layer; and, during inflammatory processes, there is infiltration by monocytes that may differentiate into a third subset of cDCs termed monocyte-derived cDCs. Tissue cDCs, such as the three mentioned, are typically referred to as 'immature', on the basis of their ability to capture antigen and their modest capacity to stimulate T cells. Upon activation, these 'immature' cells may differentiate and migrate via the afferent lymphatics into draining lymph nodes. Upon maturation, cDCs downregulate their ability to capture antigen and now possess an enhanced ability to stimulate T cells. Secondary lymphoid tissues, such as lymph nodes and spleen, therefore contain migratory tissue cDCs that have been stimulated to 'mature', but they also contain resident populations of cDCs that have the ability to capture and process internalized antigen. Unique functions have been ascribed to distinct populations of cDCs; however, the overlapping roles and diversity of responses are more complex than a simple

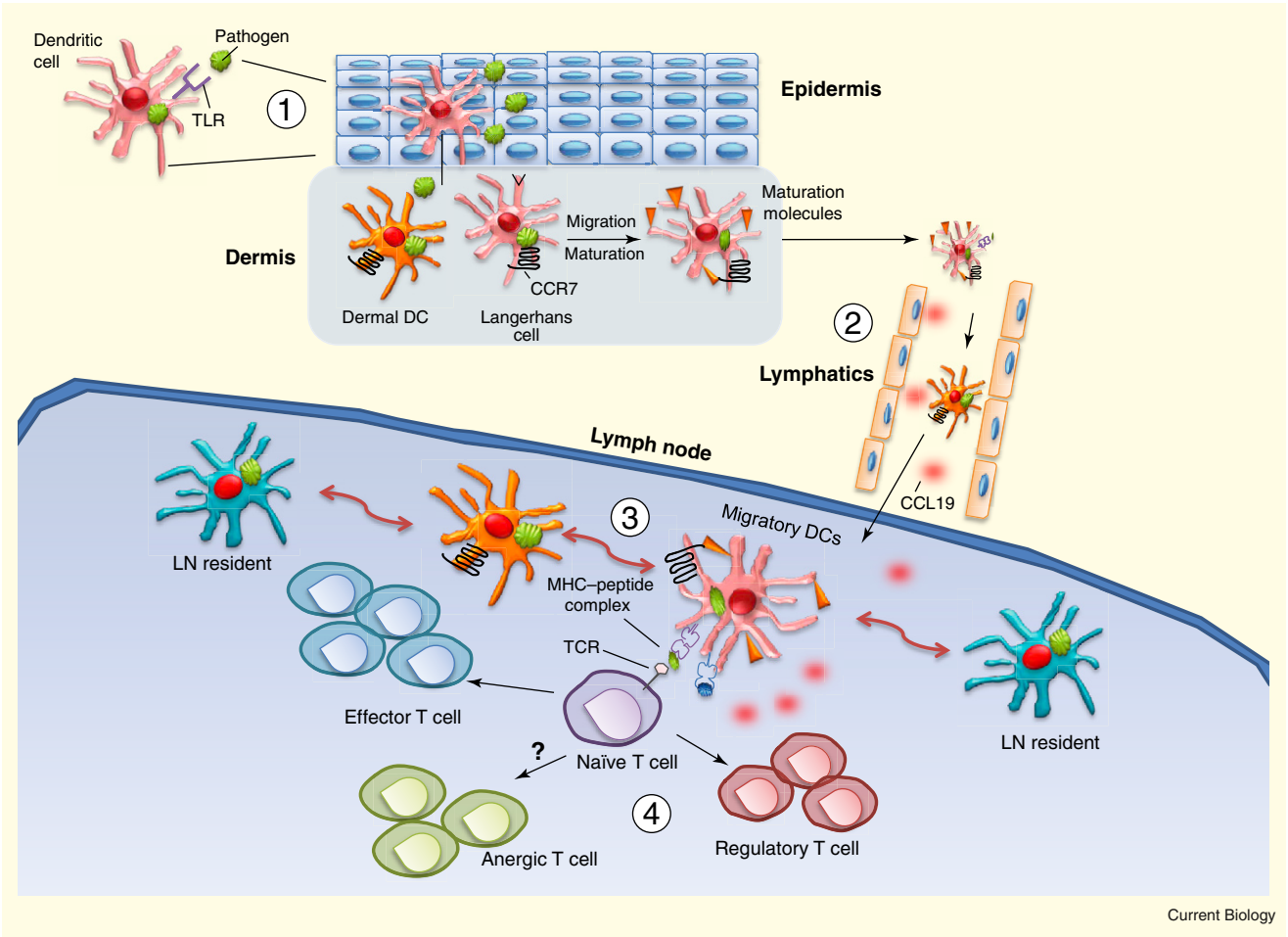

Figure 1. Schematic of the life cycle of dendritic cells, involving interaction with antigen, maturation and migration, and T-cell priming.

Tissue-resident DCs encounter pathogens in the periphery (1). Inflammatory signals produced in response to recognition of PAMPs engender changes in dendritic cells, including the expression of molecules involved in migrating out of tissues, chemokine receptors and co-stimulatory molecules. Dendritic cells migrate into the lymphatics (2) and follow chemokine gradients, specifically of the CCR7 ligands CCL19 and CCL21, to the T-cell zone of the draining lymph node. Migratory dendritic cells present MHC-I–peptide and MHC-II–peptide complexes derived from cellular and pathogen-associated proteins (3). Lymph node (LN) resident dendritic cell populations process and present antigen that is acquired in the lymph node or transferred from migratory dendritic cells. Multiple potential outcomes may result from interactions between naïve T cells and dendritic cells (4), including the generation of 'effector cells' that develop full $CD8^+$ T-cell activity or cytokine production potential, 'regulatory' cells that have the ability to modulate the function of other effector cells, and 'anergic' cells that are hyporesponsive to antigen and participate in maintaining peripheral tolerance.

'1 dendritic cell → 1 function' paradigm. Current efforts in this area reveal a coordinated exchange between multiple dendritic cell subsets throughout the course of an immune response (Figure 1). (For an in-depth review of the development of dendritic cell subsets, see Shortman and Naik (2007).)

pDCs represent the second major category of dendritic cells and were originally called interferon-producing cells as they are responsible for the robust production of type I interferons (IFNs). pDCs circulate through the blood, lymph nodes and the spleen and, upon stimulation, migrate to the T-cell area of lymphoid organs via the high endothelial venules. In addition, chronic immune activation may result in the recruitment of pDCs to non-lymphoid tissues. While most studies focus on the role pDCs play in bridging innate and adaptive immune responses in the context of systemic viral or bacterial infections, there are also reports of pDCs exerting a tolerogenic effect on the immune system. Although pDCs are interesting in their own right, this primer will focus on the role of cDCs. (For additional information on pDCs, see Gilliet *et al.* (2008).)

Sensing the microbial biosphere

Tissue cDCs are an integral part of the innate (non-specific) immune response to invading pathogens. Their presence in resting tissue, and specifically their access to the outside environment, enables immature cDCs to sense microbial organisms. While not unique in their expression of pattern recognition receptors (PRRs), much attention is afforded to how cDCs utilize these receptors for engaging pathogen-associated molecular patterns (PAMPs), such as viral and bacterial nucleic acids, and repetitive elements within the viral envelope or bacterial cell wall. The most well-studied PRRs in humans and mice are the Toll-like receptors (TLRs), but they also include Nod-like receptors, RIG-I-like receptors

and C-type lectins. Although each of these receptor families is activated by distinct agonists at the cell membrane or within intracellular vesicles, receptor binding initiates signaling cascades that result in cDC activation. Many of the genes that are involved in this transformation are upregulated via activation and nuclear translocation of the transcription factor NF-κB and members of the interferon regulatory factor family. Given the differential expression of TLRs on cDC subsets, we have focused on this family of receptors.

There have been 10 TLRs identified in humans (TLR1–10) and 12 in mice (TLR1–13, with TLR10 being a pseudogene). Broadly, they are grouped into the extracellular TLRs, which signal at the plasma membrane and recognize microbial components of the cell wall from fungi, yeast and bacteria, and the endosomal TLRs, which signal after internalization of the ligand and recognize viral and bacterial nucleic acids. Engagement of TLRs initiates physiological and phenotypic changes, including the production of chemokines and cytokines, many of which can have autocrine effects on the cDCs as well as paracrine effects on surrounding cell types. Differential expression of TLRs on cDC populations illustrates a molecular segregation of roles for the different subsets. For example, murine CD8α^+ cDCs express TLR3 but not TLR5 and are thus able to sense organisms that generate double-stranded RNA but not the flagellum proteins produced by certain bacterial strains. Human cDCs in the blood express all TLRs except for TLR9 and are complemented by pDCs, which express TLR1, 6, 7, 9 and 10.

By engaging PRR signaling pathways, DCs contribute to the innate immune response by secreting cytokines, chemokines and other bio-active molecules. Production of chemoattractants results in recruitment of neutrophils, monocytes and effector lymphocytes, as well as other circulating immune cells. Other downstream effects include increased permeability of blood vessels, which facilitates the entry of recruited cells as well as the passage of plasma components (e.g. complement and antibodies) into the site of inflammation. Local stimulation of resident lymphocytes, such as $\gamma\delta$ T cells, natural killer T cells and mucosal-associated invariant T cells, can also occur.

PRR activation can also permit the induction of anti-microbial effectors. While monocytes and macrophages are the predominant source of reactive oxygen and nitrogen species, dendritic cells do contribute to the initial innate control of pathogens. Specifically, interleukin-12 (IL-12) production by dendritic cells facilitates activation of natural killer cells, which are thought to be responsible for the first wave of IFNγ secretion. Such feed-forward pro-inflammatory cytokine loops are implicated in the early response to viruses and bacterial infection.

Bridging the innate and adaptive immune response

In addition to innate immune programs, PRR activation facilitates cDCs in their ability to orchestrate adaptive (antigen-specific) immune responses. Three critical steps in this process are: capture of antigen; migration from the tissue into the T-cell areas of lymphoid structures; and the presentation of internalized antigen on MHC molecules. Strikingly, TLR activation has been implicated in all three of these processes.

Although a hallmark of cDC maturation is the downregulation of endocytosis and phagocytosis, recent observations indicate that there is a transient increase in antigen uptake after TLR activation. Mechanistically, the increase in antigen uptake is associated with membrane-ruffling activity. Although TLRs may increase antigen capture, these receptors are not themselves capable of antigen internalization. Instead unique receptors enable the internalization of various potential sources of antigen, such as soluble protein, immune complexes, exosomes and dying cells. Regarding the specific receptors utilized for internalization, we again bump into the question of cDC subsets.

Members of the Fc receptor (FcR) family, which bind to antibodies attached to invading microbes or infected cells, are differentially expressed on both human and mouse cDC subsets, thus distinguishing their handling of immune complexes undergoing internalization. For example, human blood cDC populations differ in their expression of the FcRs CD32, CD64 and FcεRI. Also, like other human cDC subsets, Langerhans cells express CD32 constitutively; however, in the presence of IL-4 they can be induced to express CD23b. Various C-type lectins and integrin receptors, which are both implicated in antigen capture, are also differentially expressed. Clec9A and $\alpha_V\beta_5$ integrin are interesting examples, as their expression on the CD8$^+$ cDC subset in mice and BDCA-3$^+$ cDCs in humans may contribute to the efficiency of these cells in the capture of dying cells.

A unique characteristic of tissue-resident cDCs is their ability to migrate via the afferent lymphatics into the T-cell zone of the lymph node. This highlights a major difference between cDCs and macrophages, as macrophages cannot migrate out of inflamed tissues. The ability of cDCs to migrate from the site of infection to the draining lymph node is controlled by expression of chemokine receptors on their surface. Again, we find an important role for PRRs in stimulating cDC migration. For example, following NF-κB activation, the chemokine receptor CCR7 is upregulated on maturing DCs, directing movement along gradients of its chemokine ligands CCL19 and CCL21 that are established by high endothelial venules and stromal cells within the lymph node.

To say that PRRs are preparing cDCs for engagement of T cells is an oversimplification of the biology. In fact, activation of NF-κB results in the differentiation of cDCs into what could be considered a distinct cell type, with transcriptional changes on the order of thousands of genes and as yet unknown translational and post-translational modifications. In addition to stimulating a transient wave of antigen uptake and enabling cell migration, many other alterations facilitate the processing and presentation of antigen. Critical for antigen processing are: activation of pathways involved in acidification and alkylation of endosomes and phagolysosomes; protease activation, which mediates not only antigen processing but also regulates loading of MHC class II molecules; and the formation of dendritic cell aggresome-like induced structures and the modification of ubiquitin conjugation.

Orchestrating the adaptive immune response

In considering the role of cDCs in engaging the adaptive immune

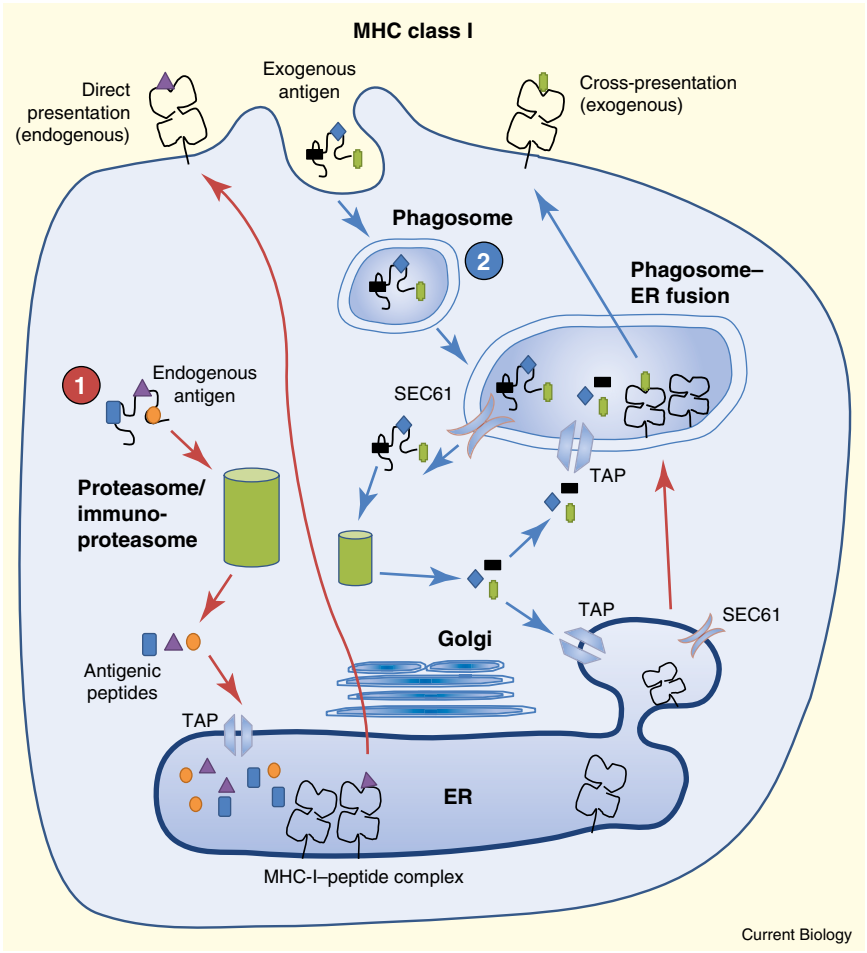

Figure 2. Major pathways involved in generating MHC-I–peptide complexes from endogenous and exogenous antigen.

MHC class I presentation of peptides derived from endogenous antigens is termed 'direct presentation' (pathway 1, red arrows). This process involves digestion of proteins in the cytosol (cellular or pathogen-derived) by the proteasome or immunoproteasome complex. These peptides are transferred into the endoplasmic reticulum (ER) where they are bound to MHC class I molecules and shuttled through the Golgi complex to the cell surface. Cross-presentation of antigen is the process whereby epitopes from exogenous antigen are bound to MHC class I molecules (pathway 2, blue arrows). Exogenous antigen is phagocytosed by the cell and the phagosome is fused with vesicles derived from the ER. Phagocytosed proteins are retrotranslocated via SEC61 out of the phagosomes into the cytosol where they can be degraded by proteasomes. Processed antigens are transported via the transporter associated with antigen processing (TAP) back into the phagosome–ER fusion where they are bound to MHC class I molecules and shuttled to the cell surface.

system, it is important to note that, in many regards, they are not unique. All nucleated cells have the capacity to present antigen on MHC class I molecules via the endogenous pathway and part of the definition of an APC is the ability to present antigen on MHC class II molecules via the exogenous pathway (Figure 2). These two pathways have been considered to be the cornerstone for immune surveillance of intracellular and extracellular microorganisms, respectively. Regarding the unique properties of cDCs, it warrants mentioning that, in addition to the expression of conventional proteasome subunits that are important for MHC class I antigen processing, some cDC subsets constitutively express alternative subunits, such as LMP2, LMP7 and MECL-1. These molecules define the consensus motifs for protein cleavage by the immunoproteasome, and, as a result, generate a distinct repertoire of peptide epitopes for presentation by cDCs, which may be important in inflammatory situations, assuming that immunoproteasome expression has been triggered in target cells. Alternatively, expression of the immunoproteasome may allow for cDCs to have their own relationship with a subset of antigen-specific T cells. Unique aspects are also involved in the presentation of exogenous antigen. For example, cDCs and macrophages show differences in the timing of phagosome acidification, which influences the balance between antigen processing and protein degradation. This translates into an increased efficiency of generating MHC class II–peptide complexes within cDCs.

Two other antigen presentation pathways have been identified. cDCs are capable of processing and presenting internalized exogenous antigen onto their own MHC class I molecule. While initially overlooked as a minor pathway, it is now clear that this mechanism accounts for the presentation of antigens derived from many viruses that do not themselves infect hematopoietic cells, antigens that are unique to cells undergoing malignant transformation or donor cells from organ transplantation, and self antigen that is restricted in its expression to non-hematopoietic cells. This process is termed 'cross-presentation', for crossing the classically defined restriction of presenting only endogenously produced antigen on MHC class I molecules. Certain cDC subsets are more efficient in their presentation of exogenous antigen, although arguably there may be an as yet unappreciated complexity regarding the source of the antigen (e.g. immune complexes versus dying cells) and the receptors involved in internalization. It should not be overlooked that other cell types, such as endothelial cells, have the capacity to cross-present antigen. Finally, there is the direct presentation of endogenous antigen onto MHC class II molecules (Figure 3). Recent work has established an important role for autophagy in the delivery of cytosolic antigen into the vesicular compartment of antigen-presenting cells. Although cDCs utilize this mechanism, it is too early to say whether they are unique in this regard.

Form does not always dictate function and just because a cell presents antigen does not mean it can initiate a T-cell response. Indeed, this is where cDCs rise high above all other

cell types described and earn their title as the 'professional antigen-presenting cell'. This term was first coined by Polly Matzinger for the ability of cDCs to activate naïve T cells, distinguishing cDCs from macrophages and B cells, which are capable of presenting antigen and re-stimulating memory T cells but are not capable of initiating a priming response. Arguably, cDCs may also be unique in their ability to initiate peripheral tolerance to a subset of tissue-restricted antigens. This latter assertion is based on their ability to capture antigen and migrate to the T-cell areas of draining lymph organs for the engagement of naïve T cells. As naive T cells do not enter resting peripheral tissue, this may be the only means of inactivating potentially self-reactive tissue-specific T cells.

So, what are the rules for dictating the outcome of priming versus tolerance? First, some definitions: T-cell priming is the result of a series of events that include T-cell receptor engagement, cell division and the acquisition of effector function (e.g. cytokine production, cytotoxicity); tolerance also involves T-cell receptor engagement and it may in some cases include cell division (especially for $CD8^+$ T cells), but typically these cells do not acquire effector function, instead undergoing a programmed cell death. In the literature, there is much discussion of T-cell anergy being a mechanism or state of tolerance (defined by cells doing nothing); however, in many cases it seems that such anergic cells do in fact have regulatory functions. For the purposes of this discussion, we will avoid the term 'activation' for describing lymphocytes as it does not properly specify the outcome of antigen presentation and T-cell engagement.

The first rule of T-cell receptor engagement is a requirement for MHC–peptide complexes on an antigen-presenting cell (referred to as 'signal 1'). The second rule concerns co-stimulatory molecules, such as B7-family members (e.g. CD80, CD86) on cDCs, engaging CD28 on T cells (referred to as 'signal 2'). Prior models suggested that signal 1 in the absence of signal 2 results in tolerance, and that signal 1 in the presence of signal 2 confers the ability to prime an adaptive immune response. As such, immature cDCs were thought to induce tolerance and, upon maturation, which results in the upregulation of co-stimulatory molecules, cDCs would be responsible for priming.

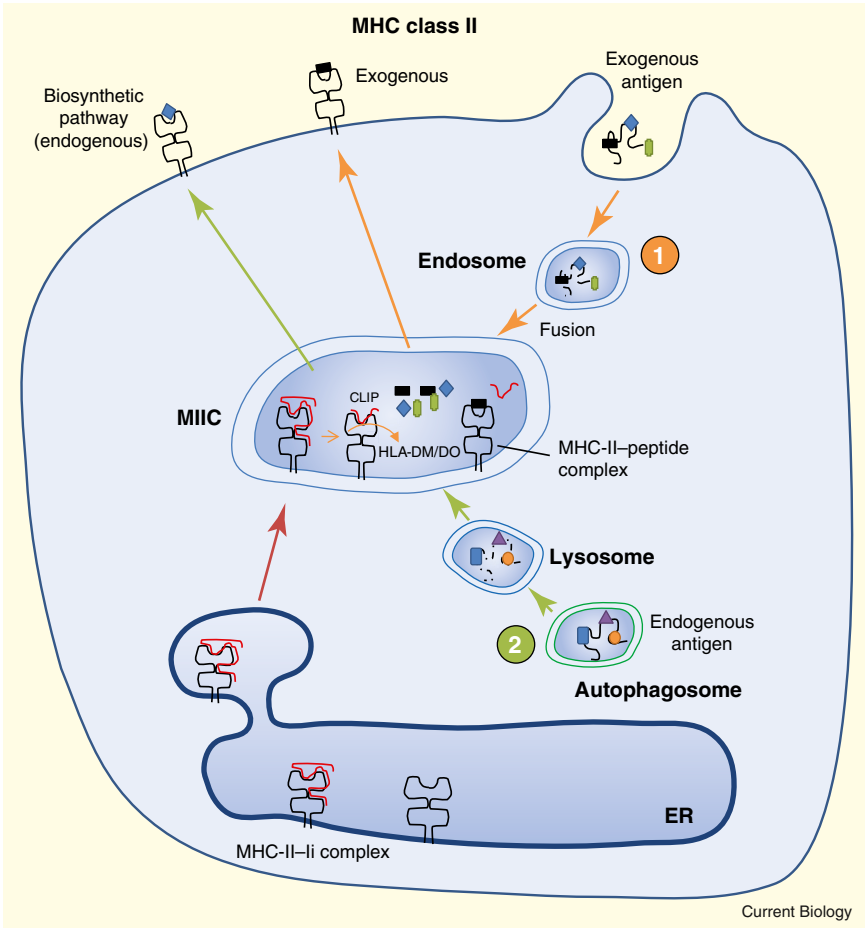

Figure 3. Major pathways involved in generating MHC-II–peptide complexes from endogenous and exogenous antigen.

For MHC class II presentation of peptides derived from exogenous antigens (pathway 1, orange arrows), exogenous antigen is endocytosed into the cell where it is partially digested and incorporated into the MHC-II compartment (MIIC). Potential epitopes compete with class-II-associated invariant chain (Ii) peptide (CLIP) for binding to the MHC class II molecule (with HLA-DM/DO facilitating the removal of CLIP) before transport to the cell surface. The biosynthetic pathway of loading MHC class II molecules with endogenous antigens (pathway 2, green arrows) involves the formation of MHC-II–peptide complexes where the peptide antigen is derived from endogenous proteins. This pathway is initiated by encapsulation of cytosolic components (including cellular and pathogen-derived material) by autophagosomes. These vesicles are fused with lysosomes, which digest the material, generating potential antigenic epitopes that are transported into the MIIC and loaded onto MHC class II molecules.

Recent studies indicate that this model is flawed in several regards. Given that naïve T cells do not traffic into resting tissue, tolerance to self-antigen must occur in lymphoid organs. As such, cDCs are required to present that antigen and careful cell biological studies indicate that maturation is a pre-requisite for MHC–peptide presentation of tissue-restricted antigen. Further, to reach the T-cell area, cDCs must upregulate CCR7. Finally, it has been demonstrated that, in many experimental systems, co-stimulatory molecules are required for T-cell tolerance. While the details may differ for constitutively expressed versus tissue-restricted antigen, it seems that TLR activation of cDCs does not determine the outcome of an immune response.

Complementing these findings, it has been described that cDCs require helper signals in order to effectively prime $CD8^+$ T cells. Such helper signals include CD40L, delivered by antigen-specific $CD4^+$ T cells, which in turn stimulates the production of pro-inflammatory factors, collectively referred to as 'signal 3'.

Mechanisms of immune evasion

Due to the pivotal role that dendritic cells have in coordinating the innate and adaptive immune responses, it is no surprise that pathogens have evolved to subvert the role of these cells in host immunity. Here, we provide examples of the diverse and creative strategies adopted by microbes to kill, avoid, hijack and selectively suppress DC functions. Collectively, these various pathogen-associated countermeasures have the capacity to subvert pathogen clearance by antigen-specific T cells.

During infection, the measles virus engages cDCs in the respiratory mucosa and lymphoid organs. A transient but profound immunosuppression is observed that occasionally results in opportunistic infections that are responsible for a high rate of mortality in children. Strikingly, infection of cDCs with measles virus results in syncitia formation, which supports high replication of the virus. More disturbingly, this virus also results in the profound death of immature DCs, possibly via upregulation of the interferon-induced, pro-apoptotic protein TRAIL. Although these phenomena account for early immunosuppression, in surviving hosts dendritic cells ultimately support the priming of virus-specific T cells that mediate viral clearance.

Another mechanism of viral immune evasion is characterized by Herpesviridae. Members of this family have evolved decoy mechanisms that allow them to avoid opsonization and uptake by cDCs. These mechanisms include the production of viral proteins that bind complement and antibodies, thus blocking receptor-mediated phagocytosis. In addition, expression of viral cytokines and viral chemokines or chemokine receptor antagonists participates in the skewing of responses to favor viral latency and prevent clearance.

Pathogens can also use cDCs to mediate their dissemination. An example of this is the ability of *Toxoplasmosis gondii* to infect cDCs in the intestinal mucosa and alter their migratory properties. The aberrant trafficking of cDCs is facilitated in part by making them insensitive to chemokine gradients and also by enhancing their ability to cross endothelial barriers. Via as yet unknown circulatory pathways, parasitized cDCs are thought to be responsible for delivering *T. gondii* to the brain. Interestingly, similar strategies may be involved in the dissemination of prions.

Many bacteria have been reported to subvert cDC function with *Mybacteria ulcerans* being one important example. Through the production of mycolactone, this pathogen inhibits cDC maturation and blocks the production of critical pro-inflammatory chemokines.

Maintenance of peripheral tolerance

Although it may be tempting to focus solely on the responsibilities that dendritic cells have in orchestrating responses involved in pathogen recognition and clearance, these cells also play an essential role in maintaining peripheral tolerance to self-antigens. One important mechanism may include the normal turnover that occurs in many tissues and allows immature dendritic cells, in the steady state, to capture self-antigen from internalized dying cells and, upon maturation, migrate to draining lymph nodes and induce tolerance in self-reactive T cells. In the case of $CD4^+$ T cells, this may result in the establishment of regulatory cells, and, for $CD8^+$ T cells, tolerance is contingent on the absence of helper signals. Current views suggest that 'tolerogenic dendritic cells' have the capacity to present antigen and engage T cells, but do not express inflammatory mediators.

Clinical use

As we learn more about the function of cDCs in orchestrating immunity, it becomes apparent that we can also put them to work for our own therapeutic interests, especially in situations where they may have been outwitted by self cells undergoing malignant transformation or by invading microorganisms. Indeed, many therapies have been envisioned to harness the selectivity of cDC–T-cell engagement with the idea of arming the adaptive immune system to respond to cancer and infected cells. We briefly highlight three different approaches to utilizing cDCs in clinical trials.

First, cDCs may be isolated and/or expanded *ex vivo*, allowing loading of the cDC with target antigen. In cases where the antigen and specific MHC epitopes have been defined, loading may involve pulsing with protein, peptide or transfection with antigen-encoding nucleic acids. Alternatively, cDCs can be loaded with dying cells, exosomes, complexes of antigen and heat shock protein, or cell lysates. These approaches are attractive as they have the benefit that the precise antigen or epitope does not need to be known. The cDC does the work in processing and presenting the tumor or microbial-associated antigen that fits that individual's MHC haplotype. *Ex vivo* maturation and adoptive transfer of the antigen-bearing cDCs to recipients will ideally prime T-cell responses that are capable of rejecting tumor cells and clearing infectious agents.

A second approach is aimed at *in vivo* targeting of cDCs. In these approaches, known antigens may be complexed with targeting vectors, such as antibodies selective for cDC receptors or viral vectors with tropism for cDCs. Finally, the injection of DNA or nanoparticles may favor uptake and presentation by cDCs.

Finally, there have been efforts to alter the microenvironment in which cDC and T-cell interactions occur, predicated on the belief that tumor or microbial antigens are being presented but not in a context that is conducive for T-cell priming. The alterations to the microenvironment may be achieved by influencing three stages of cDC differentiation and function: certain growth factors are capable of selectively differentiating precursors toward the DC lineages; chemokines have been utilized to recruit distinct cDC subsets to the inflammatory site; and, by injection of immune adjuvants or 'signal 3' stimuli, there is the hope of promoting effector T-cell priming.

With each of these cDC-based therapies there are pitfalls and obstacles that have prevented our ability to harness their full potential. Through a better understanding of disease pathogenesis and optimal vaccine strategies, there remains the hope of increased clinical applications for immune-based treatment.

Future directions

The purpose of this primer was to outline some of the basic characteristics and functions of cDCs and how they are involved in innate and adaptive immune responses. Our understanding of

dendritic cell biology is increasing at a rapid pace. Areas of current interest include: the identification and characterization of new subsets of dendritic cells; advances in intra-vital microscopy, which facilitates real-time observations of cDCs with cognate T cells; evaluation of how cell death influences dendritic cell cross-priming; understanding the context in which dendritic cells may optimally facilitate the expansion or differentiation of regulatory T cells; and further identification of endogenous activators responsible for dendritic cell maturation in the absence of PAMPs. These areas of research (and many others) hold great promise to advance our understanding of how dendritic cells orchestrate the immune response in health and pathology.

Further reading

Albert, M.L. (2004). Death-defying immunity: do apoptotic cells influence antigen processing and presentation? Nat. Rev. Immunol. *4*, 223–231.

Callahan, M.K., Garg, M., and Srivastava, P.K. (2008). Heat-shock protein 90 associates with N-terminal extended peptides and is required for direct and indirect antigen presentation. Proc. Natl. Acad. Sci. USA *105*, 1662–1667.

Coutanceau, E., Decalf, J., Martino, A., Babon, A., Winter, N., Cole, S.T., Albert, M.L., and Demangel, C. (2007). Selective suppression of dendritic cell functions by Mycobacterium ulcerans toxin mycolactone. J. Exp. Med. *204*, 1395–1403.

Gilliet, M., Cao, W., and Liu, Y.J. (2008). Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. Nat. Rev. Immunol. *8*, 594–606.

Hege, K.M., Jooss, K., and Pardoll, D. (2006). GM-CSF gene-modifed cancer cell immunotherapies: of mice and men. Int. Rev. Immunol. *25*, 321–352.

Kawai, T., and Akira, S. (2007). Signaling to NF-kappaB by Toll-like receptors. Trends Mol. Med. *13*, 460–469.

Merad, M., Ginhoux, F., and Collin, M. (2008). Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. Nat. Rev Immunol. *8*, 935–947.

Reis e Sousa, C. (2006). Dendritic cells in a mature age. Nat. Rev. Immunol. *6*, 476–483.

Shortman, K., and Liu, Y.J. (2002). Mouse and human dendritic cell subtypes. Nat. Rev. Immunol. *2*, 151–161.

Shortman, K., and Naik, S.H. (2007). Steady-state and inflammatory dendritic-cell development. Nat. Rev. Immunol. *7*, 19–30.

Steinman, R.M., and Cohn, Z.A. (1973). Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J. Exp. Med. *137*, 1142–1162.

Tacken, P.J., de Vries, I.J., Torensma, R., and Figdor, C.G. (2007). Dendritic-cell immunotherapy: from ex vivo loading to in vivo targeting. Nat. Rev. Immunol. *7*, 790–802.

Uhl, M., Kepp, O., Saklani, H., Vicencia, J.M., Kroemer, G. and Albert, M.L. (2009). Autophagy within the antigen donor cell facilitates efficient antigen cross-priming of virus-specific CD8+ T cells. Cell Death Differentiation, in press.

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A simple non-specific chemical signal mediates defence behaviour in a specialised ant–plant mutualism

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Specialist ‘plant-ants’ defend their ant-plant hosts from herbivores in exchange for rewards, including shelter and food [1]. Many of these symbiotic associations are obligate mutualisms, in which ant fitness is strongly tied to host protection. Protection should be enhanced by efficient detection of attacking herbivores [1–3]. How information about herbivore presence could be communicated from plant to ant has been little studied. In several systems, plant extracts have been shown to induce increased ant patrolling [2,3], but the compounds eliciting ant defence have never been identified. We have characterized the volatile compounds emitted by damaged leaves of a specialized ant-plant and demonstrated in field experiments the identity of chemicals that induce plant-protective behaviour.

The ant *Petalomyrmex phylax* protects *Leonardoxa africana africana* against herbivores [4]. Young leaves are constantly patrolled; ants visit mature leaves to harvest extrafloral nectar [4]. Some volatile organic compounds (VOCs), including methyl salicylate, are emitted constitutively by young leaves but not mature leaves [2]. Methyl salicylate was not detected in 30 minute hexane washes of mature leaves [2], but small amounts were detected in 2 hour extracts, indicating that this compound is less concentrated, perhaps also differently distributed, in mature leaves. We showed that *P. phylax* exited from domatia — sites on the host plant adapted to house the ants — more rapidly and in greater numbers when an experimentally damaged mature leaf was placed near the entrance of the domatium than when an untreated filter paper (n = 31) or an intact mature leaf (n = 10) was placed in the same position ($P < 0.001$ in all cases) (see Supplemental data available on-line with this issue for details). The host-protective behaviour of *P. phylax* is thus mediated by chemical signals, as would be expected from theory and as has been demonstrated in many cases [2,3,5]. In contrast, for *Cataulacus mckeyi*, a specific ant parasite of this mutualism [4], we showed that experimentally damaged leaves held at the entrance of domatia did not induce exit of more ants than did filter paper (n = 11). Moreover, significantly greater numbers of workers of the mutualist *P. phylax* exited domatia in the same situation, reflecting variation in the investment in protection in different plant-ants associated with the same host species, a phenomenon that has been reported in only a few other cases [3].

Signalling herbivore activity confers the greatest advantage on a plant when the signals reach the ants rapidly; selection should thus have favoured the production of rapidly diffusing compounds. We detected three compounds in VOC emissions from all experimentally damaged *L. a. africana* mature leaves: methyl salicylate, 2E hexen-1-ol, and hexanal. These common green leaf volatiles are known to play roles in the attraction to the plants that produce them of natural enemies of phytophagous insects [6]. They have also been reported to be present in emissions from damaged leaves of several Asian *Macaranga* spp. ant-plants [7].

To determine the biologically active compounds, we compared the reactions of *P. phylax* to different stimuli (filter papers impregnated with synthetic compounds) placed near domatia entrances (Figure 1). We found that the number of ants exiting domatia was significantly higher for methyl salicylate, 2E hexen-1-ol and the mix than for hexanal alone and control (Figure 1). There was no significant difference between methyl salicylate, 2E hexen-1-ol and the mix, nor between hexanal and control,